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## **Divergent memory B cell responses in a mixed infant pneumococcal conjugate vaccine schedule**

Trück, Johannes ; Mitchell, Ruth ; Jawad, Sena ; Clutterbuck, Elizabeth A ; Snape, Matthew D ; Kelly, Dominic F ; Voysey, Merryn ; Pollard, Andrew J

**Abstract:** **BACKGROUND:** Vaccine-induced immunity against pneumococcal infection relies on the generation of high concentrations of antibody and B-cell memory. Both the 10- and the 13-valent pneumococcal conjugate vaccines (PCV-10 and PCV-13) effectively reduce disease caused by vaccine serotypes. It is unknown whether the generation of B-cell memory requires several doses of the same vaccine or whether different PCVs are interchangeable. **METHODS:** Children in the UK (n=178) who had previously received PCV-13 at 2 and 4 months were randomized 1:1 to receive a PCV-13 or PCV-10 booster at age 12 months. Peripheral blood memory B cells (BMEM) were quantified before and at 1 and 12 months following vaccination using a cultured ELISpot assay for pneumococcal serotypes 1, 3, 4, 9V, 14, 19A and diphtheria and tetanus toxoid. Correlations between BMEM frequencies and simultaneously measured antibody (IgG and OPA) was also assessed. **RESULTS:** A significant rise in post-booster BMEM frequency was seen for 5 out of six serotypes in the PCV-13 group and none in the PCV-10 group. In the PCV-13 group, there was a particularly large increase in serotype 3-specific BMEM associated with only a small increase in antibody. Post-booster BMEM responses correlated positively with antibody, but correlations between pre-booster BMEM and subsequent BMEM and antibody responses were inconsistent. **CONCLUSIONS:** Following priming with PCV-13 in early infancy, a booster dose of PCV-10 does not induce detectable peripheral blood BMEM responses but a PCV-13 booster does induce robust responses. Booster responses to pneumococcal conjugate vaccines may be dependent on homologous carrier protein priming.

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# Divergent Memory B Cell Responses in a Mixed Infant Pneumococcal Conjugate Vaccine Schedule

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**Background:** Vaccine-induced immunity against pneumococcal infection relies on the generation of high concentrations of antibody and B cell memory. Both the 10- and the 13-valent pneumococcal conjugate vaccines (PCV-10 and PCV-13) effectively reduce disease caused by vaccine serotypes. It is unknown whether the generation of B cell memory requires several doses of the same vaccine or whether different PCVs are interchangeable.

**Methods:** Children in the United Kingdom (n=178) who had previously received PCV-13 at 2 and 4 months were randomized 1:1 to receive a PCV-13 or PCV-10 booster at age 12 months. Peripheral blood memory B cells ( $B_{MEM}$ ) were quantified before and at 1 and 12 months after vaccination using a cultured enzyme-linked immunospot assay for pneumococcal serotypes 1, 3, 4, 9V, 14, 19A, and diphtheria and tetanus toxoid. Correlations between  $B_{MEM}$  frequencies and simultaneously measured antibody (IgG and opsonophagocytic assay) was also assessed.

**Results:** A significant rise in postbooster  $B_{MEM}$  frequency was seen for 5 out of 6 serotypes in the PCV-13 group and none in the PCV-10 group. In the PCV-13 group, there was a particularly large increase in serotype 3-specific

$B_{MEM}$ , associated with only a small increase in antibody. Postbooster  $B_{MEM}$  responses correlated positively with antibody, but correlations between prebooster  $B_{MEM}$  and subsequent  $B_{MEM}$  and antibody responses were inconsistent.

**Conclusions:** After priming with PCV-13 in early infancy, a booster dose of PCV-10 does not induce detectable peripheral blood  $B_{MEM}$  responses but a PCV-13 booster does induce robust responses. Booster responses to PCVs may be dependent on homologous carrier protein priming.

**Key Words:** vaccination, interchangeability, pneumococcal conjugate vaccine, memory B cells

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## INTRODUCTION

Vaccination against *Streptococcus pneumoniae* with currently used 10- and 13-valent pneumococcal conjugate vaccines (PCV-10 and PCV-13) has been shown to dramatically reduce vaccine-type invasive pneumococcal disease when included in childhood immunization schedules.<sup>1–5</sup> Both vaccines are immunogenic and have been shown to induce immune memory.<sup>6,7</sup>

There is limited information on the interchangeability of PCVs; however, there are potential reasons why it may be advantageous to include PCV-10 in the infant vaccination schedule. Non-typeable *Haemophilus influenzae* (NTHi) protein D is not included in any concurrent or previously administered vaccine. Its use may, therefore, reduce the risk of immune interference and theoretically increase the immune response to the polysaccharides. Immune interference can occur if the same carrier protein is used in concurrent or sequential vaccinations and can suppress the immune response to the desired antigen.<sup>8</sup> In addition, use of NTHi protein D as a carrier protein may confer additional protection against NTHi infection, a common cause of otitis media in children,<sup>9</sup> although effectiveness of PCV-10 against NTHi carriage or disease has not been demonstrated in previous vaccine trials.<sup>10–12</sup>

Ideally, a vaccine should provide long-lasting protection in the form of antibodies, but should also induce immunological memory through the formation of memory B cells ( $B_{MEM}$ ). A number of investigators have studied this outcome by measuring  $B_{MEM}$  frequency in peripheral blood after vaccination.<sup>13–16</sup> The majority of  $B_{MEM}$  is thought to reside in lymphoid tissue, but small numbers of circulating  $B_{MEM}$  can be detected in peripheral blood even years after vaccination. The frequency of these cells in peripheral blood rises shortly after vaccination, particularly after booster doses.<sup>17</sup> Re-encounter with antigen triggers the proliferation of  $B_{MEM}$  and their differentiation into antibody-secreting plasma cells, giving rise to a rapid and effective secondary immune response. The presence of  $B_{MEM}$  may also contribute to the maintenance of circulating antibody by intermittent or continuous differentiation into antibody-secreting plasma cells in response to antigen-dependent or independent stimulation.<sup>18</sup>

This study evaluated the potential for the use of PCV-10 as a booster after priming with PCV-13 in infancy by assessing non-inferiority of the proportions of participants with postbooster IgG

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J.T. and R.M. contributed equally to this study.

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A.J.P. has previously conducted studies on behalf of Oxford University funded by vaccine manufacturers, including the present study, but currently does not undertake industry funded clinical trials. A.J.P. chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunisation (JCVI); the views expressed in this manuscript do not necessarily reflect the views of JCVI or DH. M.D.S. acts as chief or principal investigators for clinical trials conducted by the University of Oxford, sponsored by vaccine manufacturers, but receives no personal payments from them. M.D.S. has participated in advisory boards and industry-sponsored symposia for vaccine manufacturers, but receives no personal payments for this work. M.D.S. and J.T. have received financial assistance from vaccine manufacturers to attend scientific conferences. D.G./L.R.'s laboratory performs contract serology and receives research funding from the manufacturers of pneumococcal vaccines. D.G. acts occasionally as an advisor to GSK and other vaccine manufacturers, is an NIHR Senior Investigator, and is supported by the NIHR BRC at Great Ormond Street. The other authors have no conflicts of interest to disclose.

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$\geq 0.35$   $\mu\text{g/mL}$  for PCV-10 serotypes. The results of antibody measurements in this cohort of children have already been published and showed that in PCV-13-primed infants, a booster dose of PCV-10 induces a strong antibody response, which is generally less pronounced than the response after a PCV-13 booster.<sup>19</sup> In the analysis presented here, we investigated the frequencies of peripheral blood  $B_{\text{MEM}}$  before and at 1 and 12 months after a 12-month booster dose of PCV-10 or PCV-13. Using a cultured enzyme-linked immunospot (ELISpot) assay, we studied the  $B_{\text{MEM}}$  response to 2 different pneumococcal vaccines, of which only one had previously been given to study participants. By comparing the booster response to the 2 vaccines, which differ in the concentrations of pneumococcal polysaccharides, the method of conjugation and the type and concentrations of the carrier proteins, we had the unique opportunity to study the effects of a mixed vaccination schedule on B cell biology.

## MATERIALS AND METHODS

### Subjects and Vaccines

Healthy children who had been vaccinated with the PCV-13 at 2 and 4 months of age were recruited as previously described<sup>19</sup> (Fig., Supplemental Digital Content 1, <http://links.lww.com/INF/C656>). Ethical approval was obtained from the Oxfordshire Research Ethics Committee (reference number 11/SC/0473), and the study was registered on Clinicaltrials.gov (registration number NCT01443416). After enrolment into the study, these children were randomized to receive either PCV-10 [Synflorix, GSK Biologicals; containing capsular polysaccharides of serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F, conjugated to NTHi protein D, tetanus toxoid (18C) or diphtheria toxoid (19F), respectively] or PCV-13 [Prevenar 13, Pfizer; containing serotypes 3, 6A and 19A in addition to PCV-10 serotypes, conjugated to diphtheria toxin mutant or cross reactive material (CRM<sub>197</sub>)]. Blood samples were taken on 3 visits: at day 0 immediately before vaccination (12 months of age) and at 1 and 12 months after vaccination (13 and 24 months of age).

### Peripheral Blood Mononuclear Cell isolation and ELISpot

The cultured ELISpot assay for the detection of antigen-specific  $B_{\text{MEM}}$  was performed as previously described.<sup>20</sup> First, peripheral blood mononuclear cells were isolated from whole blood by density gradient centrifugation with lymphoprep (Axis-Shield). Cells were then cultured for 6 days at 37°C in 5% carbon dioxide and 95% humidity with an antigen stimulation mix containing *Staphylococcus aureus* Cowan strain (Calbiochem-Novabiochem) at a 1:5000 dilution, poke weed mitogen (Sigma-Aldrich) at a final concentration of 83 ng/mL and CpG oligonucleotide (InvivoGEN) at a final concentration of 1.7  $\mu\text{g/mL}$ . After harvesting, cells were washed and seeded at  $2 \times 10^5$  viable cells per well onto a 96-well plate with PVDF membranes (Millipore). Membranes were precoated with either pneumococcal polysaccharides (1, 3, 4, 9V, 14 and 19A; LCG Promochem) conjugated to methylated human serum albumin (NIBSC UK), diphtheria toxoid (10  $\mu\text{g/mL}$ , also used to represent the CRM<sub>197</sub> carrier protein; Statens Serum Institut), or tetanus toxoid (5  $\mu\text{g/mL}$ , Statens Serum Institut). Pneumococcal serotypes were chosen to reflect serotypes contained in both PCV-10 and PCV-13 (serotypes 1, 4, 9V and 14) or unique to PCV-13 (serotypes 3, 19A). Plates included phosphate-buffered saline wells and polyvalent goat anti-human immunoglobulin (10  $\mu\text{g/mL}$ ) wells as negative and positive controls, respectively. After overnight incubation, plates were washed, and bound IgG antibody was detected using a goat anti-human IgG alkaline phosphatase conjugate (Calbiochem) and alkaline phosphatase substrate kit (Bio-Rad). Plates were dried overnight before being read using an

automated ELISpot reader (AID ELR03, AID Diagnostika). All plate readings were manually checked to exclude artifacts.

### Data Analysis

The average number of spots per well was used to calculate the number of antibody-secreting cells per million peripheral blood mononuclear cells. If any spots were observed in the control phosphate-buffered saline wells, the average count across these control wells was subtracted from the average count from each type of antigen-coated well. Wells coated with total immunoglobulin were used as positive controls. Results of zero were assigned the value of 0.625 per million peripheral blood mononuclear cells (half of the lower limit of detection). Geometric mean frequencies (GMF) and corresponding 95% confidence intervals were calculated for each group and study time point. Differences between groups and time points were investigated using 2-sample *t* tests of logarithmically transformed  $B_{\text{MEM}}$  frequencies and Satterthwaite's correction for unequal variances. Correlations between responses at different time points were assessed using the Pearson correlation coefficient.

## RESULTS

### Samples

Out of a total of 434 blood samples across both groups and the 3 study visits from the original serological analysis,<sup>19</sup> 377 (87%) samples had cells available for  $B_{\text{MEM}}$  and antibody analysis. The breakdown of samples by visit and vaccine group is shown in Table 1.

### Antigen-specific $B_{\text{MEM}}$ GMF by Antigen and Vaccine Group

At 12 months of age,  $B_{\text{MEM}}$  frequencies were low, and no significant differences between the groups were seen in  $B_{\text{MEM}}$  GMF for all antigens tested (Figure 1; Table, Supplemental Digital Content 2, <http://links.lww.com/INF/C657>). One month after booster vaccination at 13 month of age, significantly higher  $B_{\text{MEM}}$  GMF were found for serotypes 1, 4, 9V and 3 in the PCV-13 compared with the PCV-10 group, whereas  $B_{\text{MEM}}$  responses to tetanus toxoid were statistically higher in PCV-10 than in PCV-13 recipients (Figure 1; Table, Supplemental Digital Content 2, <http://links.lww.com/INF/C657>). One year after booster at 24 months of age, no significant differences were detected between the groups for any of the antigens tested (Figure 1; Table, Supplemental Digital Content 2, <http://links.lww.com/INF/C657>).

### Antigen-specific $B_{\text{MEM}}$ Frequency Fold Changes

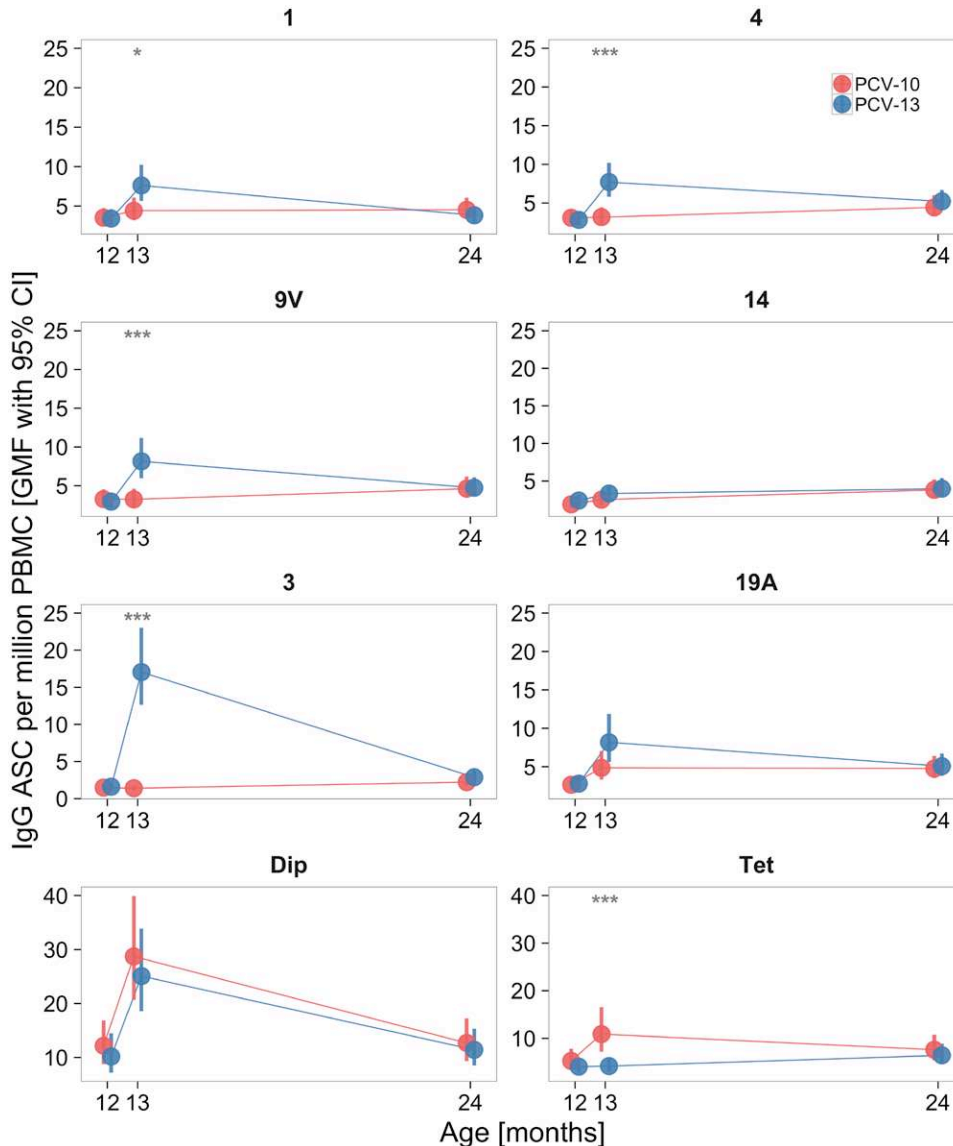
Significant increases between  $B_{\text{MEM}}$  measured at age 12 and 13 months were seen for most pneumococcal serotypes in the PCV-13 group (with the exception of serotype 14, which showed a 1.6-fold increase with a borderline *P* value of 0.05) and none in the

**TABLE 1.** Number of samples received at each visit for each vaccine group that were available for both  $B_{\text{MEM}}$  and antibody analysis

Group	Visit 1 (12 Months)	Visit 2 (13 Months)	Visit 3 (24 Months)
PCV-10	59 (67, 88%)	56 (69, 81%)	66 (75, 88%)
PCV-13	63 (73, 86%)	69 (74, 93%)	64 (76, 84%)

Values in brackets show the number of available serum samples per visit and vaccine group, as well as the percentage of samples on which both analyses were done.

$B_{\text{MEM}}$  indicates memory B cells; PCV-10, 10-valent pneumococcal conjugate vaccines; and PCV-13, 13-valent pneumococcal conjugate vaccines.



**FIGURE 1.** Geometric mean frequencies [GMF; along with 95% confidence interval (CI)] of memory B cells (B<sub>MEM</sub>) specific for pneumococcal serotypes, as well as diphtheria and tetanus toxoid. Groups were compared by independent samples *t* tests using log<sub>10</sub>-transformed data with Satterthwaite's correction for unequal variances. Stars indicate the associated *P* value (\*\*\*<0.001; \*\*<0.01; \*<0.05). 1/4/9V/14/3/19A indicates pneumococcal serotypes 1/4/9V/14/3/19A; Dip, diphtheria toxoid; PBMC, peripheral blood mononuclear cells; PCV-10, 10-valent pneumococcal conjugate vaccines; PCV-13, 13-valent pneumococcal conjugate vaccines; and Tet, tetanus toxoid.

PCV-10 group (Table 2). For antigens representing carrier proteins, a significant rise in B<sub>MEM</sub> frequencies was seen for both diphtheria and tetanus toxoid in the PCV-10 group but only for diphtheria toxoid in the PCV-13 group (Table 2). When adjusted for baseline B<sub>MEM</sub> values, age, sex and ethnicity, changes in B<sub>MEM</sub> frequencies between age 12 and 13 months were significantly greater in the PCV-13 compared with the PCV-10 group for all pneumococcal serotypes and statistically superior in PCV-10 compared with PCV-13 recipients only for tetanus toxoid (Table 2). Frequencies of B<sub>MEM</sub> were not significantly different between age 12 and 24 months for most serotypes in both groups. In the PCV-10 group, significantly higher B<sub>MEM</sub> frequencies were seen for serotypes 14 (GMF of 3.83 versus 1.91) and 19A (4.76 versus 2.66) at 24 months compared with 12 months of age, whereas in the PCV-13 group, a significant fold increase was only seen for serotype 3 (2.88 versus 1.61; Table, Supplemental Digital Content 3, <http://links.lww.com/INF/C658>). However, when comparing these fold changes from age 12 to 24 months between the vaccine groups, no differences were seen when adjusted for baseline values, age, sex and ethnicity (data not shown).

### Correlation Between Serotype-specific B<sub>MEM</sub> and Antibody Responses

Pearson correlation was used to investigate correlations between log-transformed B<sub>MEM</sub> frequencies at different time points and between B<sub>MEM</sub> and antibody responses (both IgG concentration and opsonophagocytic assay titers). All correlations and their statistical significance are shown in Figure 2. The most striking correlations were seen between B<sub>MEM</sub> and antibody, both at age 13 months. Antibody and B<sub>MEM</sub> were correlated at 12 months and at 24 months of age for some serotypes; however, this relationship was less consistent than that seen at age 13 months. B<sub>MEM</sub> at 12 and 13 months of age were also predictive of later antibody responses for some serotypes in each group, but again this relationship was not consistent across serotypes.

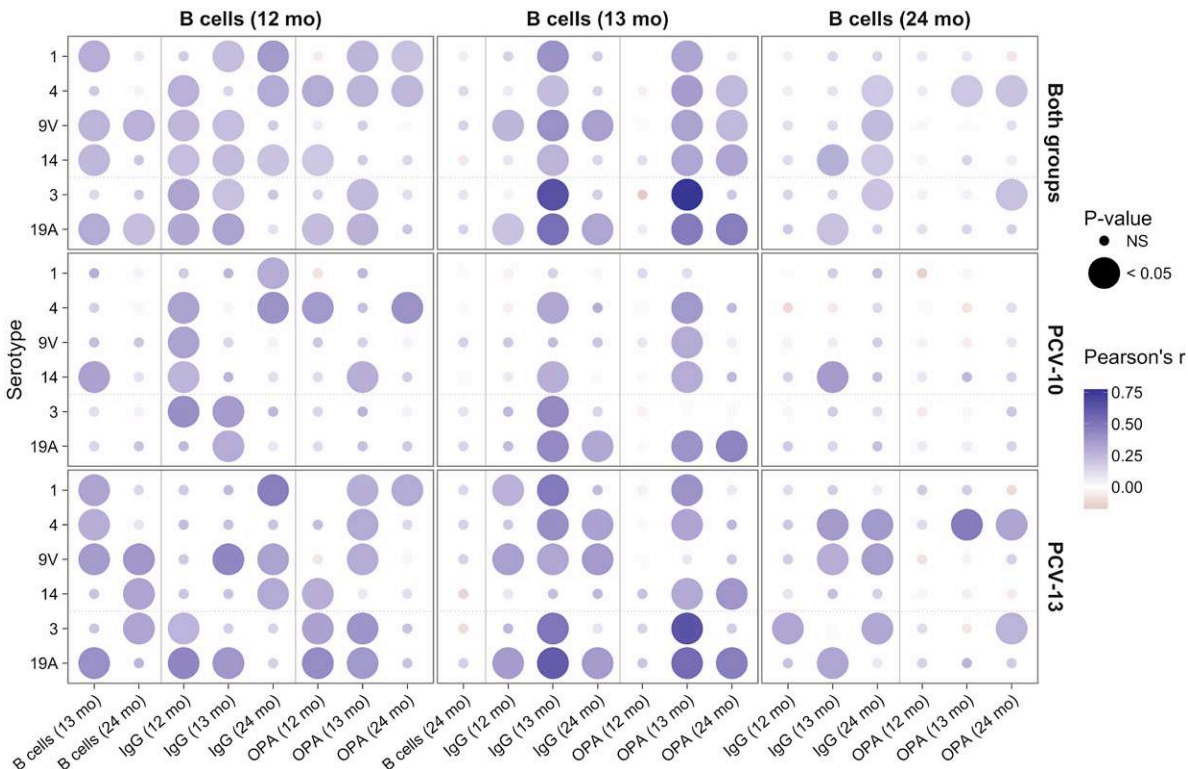
A significant increase in B<sub>MEM</sub> frequencies from 12 to 13 months of age against the majority of the tested serotypes was only observed for the PCV-13 group, but the extent of the response was serotype dependent (Table 2). In PCV-13 recipients, the immune response against serotype 3 was associated with a strong increase in peripheral B<sub>MEM</sub> (Figure 2) and associated with a weak antibody



**TABLE 2.** Antigen-specific B<sub>MEM</sub> frequency geometric mean fold change from 12 to 13 months by vaccine group

Serotype	N	PCV-10			N	PCV-13			Adjusted Treatment (Group) Effect*		
		Geometric Mean Fold Rise†	95% CI	P Value		Geometric Mean Fold Rise†	95% CI	P Value	PCV-10/PCV-13	95% CI	P Value
1	39	1.160	0.714–1.886	0.544	54	2.436	1.530–3.877	<0.001	0.469	0.284–0.773	0.003
4	39	0.929	0.563–1.532	0.770	54	2.917	1.855–4.588	<0.001	0.340	0.210–0.550	<0.001
9V	38	0.880	0.504–1.537	0.650	54	3.091	1.872–5.105	<0.001	0.295	0.172–0.506	<0.001
14	38	1.025	0.613–1.713	0.925	54	1.556	1.000–2.420	0.050	0.582	0.352–0.964	0.036
3‡	39	0.809	0.549–1.191	0.278	54	12.90	8.587–19.37	<0.001	0.057	0.037–0.086	<0.001
19A‡	38	1.775	0.996–3.161	0.051	53	3.238	1.951–5.373	<0.001	0.429	0.240–0.766	0.005
Dip§	33	2.157	1.231–3.780	0.008	51	2.660	1.568–4.510	<0.001	0.865	0.525–1.426	0.566
Tet¶	35	2.249	1.089–4.643	0.029	51	1.084	0.604–1.944	0.785	2.188	1.229–3.894	0.008

N indicates number of paired samples.  
\*Ratio of geometric mean fold rises from 12 to 13 months (PCV-10/PCV-13), adjusted for 12 month values, age, sex and ethnicity.  
†Geometric mean change from 12 months to 13 months.  
‡Serotypes contained in PCV-13 only.  
§Represents carrier protein CRM<sub>197</sub> (PCV-13) or diphtheria toxin (PCV-10).  
¶Represents carrier protein tetanus toxoid (contained in PCV-10 only).  
1/4/9V/14/3/19A indicates pneumococcal serotypes 1/4/9V/14/3/19A; B<sub>MEM</sub>, memory B cells; CI, confidence interval; Dip, diphtheria toxin; PCV-10, 10-valent pneumococcal conjugate vaccines; PCV-13, 13-valent pneumococcal conjugate vaccines; and Tet, tetanus toxoid.



**FIGURE 2.** Correlation between memory B cells (B<sub>MEM</sub>) frequency at 12, 13 and 24 months of age and B<sub>MEM</sub> frequency at other time points, IgG and OPA. NS indicates not significant; and OPA, opsonophagocytic assay.

responses compared with other serotypes.<sup>19</sup> In contrast, antibody responses against serotype 14 were strong,<sup>19</sup> whereas it was associated with only a small increase in postbooster B<sub>MEM</sub> frequency (Figure 2).

DISCUSSION

This study provides an assessment of the B<sub>MEM</sub> responses after booster vaccination with either PCV-13 or PCV-10 in children

previously primed with 2 doses of PCV-13. Our results show that PCV-13-primed children do not generate peripheral B<sub>MEM</sub> in response to a PCV-10 booster. This is a surprising finding because 4 of the 6 serotypes that were evaluated are contained in both vaccines. However, the vaccines differ in the concentrations of pneumococcal polysaccharides, the method of conjugation and the type and concentrations of the carrier proteins, which may result in diverging presentation and processing of antigens.

One recent study assessed the short-term (7–9 days after booster) immunogenicity and the plasma and  $B_{MEM}$  response around a booster dose given at 11 months of age after a vaccination series with either PCV-10 or PCV-13.<sup>21,22</sup> This study showed that the pneumococcal serotype-specific frequencies of peripheral blood  $B_{MEM}$  measured before and shortly after a PCV booster were significantly higher in PCV-13 compared with those in PCV-10 recipients for 3 out of 4 serotypes common to both vaccines.<sup>21</sup> No such differences were seen for serotype-specific plasma cell responses,<sup>21</sup> but the study also showed statistically superior postbooster IgG responses in the PCV-13 compared with the PCV-10 group to the majority of serotypes common to both vaccines.<sup>22</sup> After 4 doses of PCV-10, a significant  $B_{MEM}$  booster response was observed<sup>21</sup>; however, even in that study, PCV-13 appeared to be a more potent inducer of  $B_{MEM}$  than PCV-10. Interchangeability of PCVs was not assessed in that study because children received the same booster vaccine as they had received in infancy.

In the present clinical trial, in the PCV-10 group, no change in serotype-specific  $B_{MEM}$  frequencies was seen between before booster and 1 month later booster vaccination, whereas a significant increase in  $B_{MEM}$  specific for diphtheria and tetanus toxoid was noted between these 2 study time points. Children who were allocated to the PCV-10 group had previously received 2 doses of PCV-13 and were, therefore, already primed with all pneumococcal serotypes contained in PCV-10. However, their immune system had not been exposed to the same conjugates and the carrier protein D derived from NTHi in the form of a vaccine, although some may have encountered it through carriage or disease. Priming and boosting with different carrier proteins has previously been investigated in children vaccinated against meningitis C.<sup>23</sup> Children primed with a dose of tetanus toxoid–conjugated polysaccharide (MenC-TT) at 3 months generated better  $B_{MEM}$  responses to a MenC-TT booster at 12 months than those primed with either 1 or 2 doses of CRM-conjugated polysaccharide (MenC-CRM). In the present study,  $B_{MEM}$  booster responses to serotypes 18C and 19F, which are conjugated to tetanus and diphtheria toxoid, respectively, were not assessed. Investigating 18C and 19F  $B_{MEM}$  responses to a booster dose of PCV-10 in PCV-13-primed children may have provided further insight into whether exposure to the carrier protein through routine vaccination (as these children have already received several doses of diphtheria and tetanus toxoid-containing vaccines) is enough to generate pneumococcal B cell memory in these children or whether the carrier protein has to be conjugated to pneumococcal polysaccharides to achieve effective priming. In the meningitis C study, children primed with MenC-CRM generated inferior booster B cell responses to a MenC-TT booster, despite previous exposure to tetanus toxoid in other routine vaccinations,<sup>23</sup> suggesting that in children of this age group, priming with a similar carrier protein is essential for  $B_{MEM}$  generation. Overall, the findings of the present study suggest that in children previously primed with PCV-13, the protein D–conjugated polysaccharides in a booster dose of PCV-10 appear to evoke a primary rather than a secondary immune response. This may involve, in the short term, processing of antigens similar to plain polysaccharides (which do not generate  $B_{MEM}$  responses) rather than recognition as T-dependent antigens. Future studies are needed to explore in detail the observed lack of B cell responses after PCV-10 vaccination, especially by investigating responses in children primed with PCV-10 and boosted with PCV-13.

In the group of children who were primed and boosted with PCV-13, the  $B_{MEM}$  peak at age 13 months was followed by a return to almost baseline by age 24 months for most serotypes, by which time most cells have probably transited to lymph nodes. In PCV-10 recipients, previously receiving PCV-13 primary vaccination, an increase in  $B_{MEM}$  frequencies was seen between 12 and 24 months of age for all serotypes tested, which was significant for serotype 14 and serotype 19A (Table, Supplemental Digital Content 3, [\[links.lww.com/INF/C658\]\(http://links.lww.com/INF/C658\)\). These findings suggest that there was a slower generation of  \$B\_{MEM}\$  in response to PCV-10 vaccination or ongoing generation through natural exposure via carriage,<sup>24,25</sup> resulting in some increase in  \$B\_{MEM}\$  frequencies in the peripheral blood 1 year after the booster vaccination.](http://</a></p></div><div data-bbox=)

It is possible that because of differences between polysaccharides in the vaccine and those bound to the ELISpot plates, the assay used in this study was less able to detect  $B_{MEM}$  induced by the PCV-10 vaccine, despite their presence in peripheral blood. However, the fact that a late rise in  $B_{MEM}$  was detected in the PCV-10 group at age 24 months makes this explanation less likely.

In the PCV-13 group, the most marked  $B_{MEM}$  response was seen for serotype 3. However, the immune response to serotype 3 polysaccharide has previously been shown to be atypical, and antibody responses to a booster appear to be particularly impaired.<sup>26</sup> Compared with other serotypes, immunogenicity for serotype 3 is attenuated and a serotype 3–containing vaccine has previously failed to show protective efficacy against serotype 3 otitis media,<sup>9</sup> although postimplementation surveillance suggests that immunization with PCV-13 does have some efficacy against serotype 3.<sup>27</sup> Despite diminished antibody production, the  $B_{MEM}$  response to serotype 3 polysaccharide has previously been shown in adults to resemble that of other serotypes.<sup>28</sup> In the present analysis, serotype 3 showed both the greatest increase in  $B_{MEM}$  frequency and the lowest antibody response of all PCV-13 serotypes (Figure 1),<sup>19</sup> suggesting that one reason for impaired serotype 3–specific antibody concentrations after a booster might be that the immune response is driven towards the generation of  $B_{MEM}$  rather than antibody-secreting cells but still provides some protection.

A question addressed by a number of studies has been whether baseline  $B_{MEM}$  positively correlate with later antibody responses.<sup>17</sup> A correlation between  $B_{MEM}$  frequency after priming and antibody persistence at 1 year was found in children receiving the meningococcal serotype C conjugate vaccine,<sup>16</sup> and for some meningococcal serogroups in children receiving the MenACWY vaccine<sup>29</sup>; however, no relationship between  $B_{MEM}$  and antibody was found in older children receiving a booster dose of the Hib-MenC conjugate vaccine.<sup>30</sup> Here we show that  $B_{MEM}$  and antibody responses 1 month after booster correlate well for most serotypes, particularly in PCV-13 recipients; however, baseline  $B_{MEM}$  are not a consistent predictor of postvaccination  $B_{MEM}$  and IgG responses. This may reflect the unmeasured contribution of other antibody-producing cell types, such as long-lived plasma cells.

## CONCLUSION

Here we present the first study assessing the  $B_{MEM}$  response to a mixed PCV schedule. We were unable to detect serotype-specific  $B_{MEM}$  after a booster dose of PCV-10 given to children who had been primed with PCV-13. In contrast, a strong serotype-specific  $B_{MEM}$  response was generated in children primed with PCV-13 after receipt of a PCV-13 booster. These findings suggest that immunizing with a PCV containing polysaccharides conjugated to a novel carrier protein is not sufficient to generate a rapid and strong  $B_{MEM}$  response, at least when primary vaccination with PCV-13 is followed by a booster dose of PCV-10. Although the clinical implications of these results are unknown, they still indicate that a vaccination series only using PCV-13 is advantageous over a mixed PCV schedule consisting of a priming series with PCV-13 and boosting with PCV-10.

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